

AutoUnmix: an autoencoder-based spectral unmixing method for multi-color fluorescence microscopy imaging: supplement

YUAN JIANG,^{1,†} HAO SHA,^{1,†} SHUAI LIU,² PEIWU QIN,^{3,4} AND YONGBING ZHANG^{1,*}

¹*School of Computer Science and Technology, Harbin Institute of Technology, Shenzhen, Guangdong 518055, China*

²*Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen, Guangdong Province 518055, China*

³*Center of Precision Medicine and Healthcare, Tsinghua-Berkeley Shenzhen Institute, Guangdong Province, 518055, China*

⁴*Institute of Biopharmaceutical and Health Engineering, Tsinghua Shenzhen International Graduate School, Shenzhen, Guangdong Province, 518055, China*

[†]*The authors contributed equally to this work.*

^{*}*ybzhang08@hit.edu.cn*

This supplement published with Optica Publishing Group on 22 August 2023 by The Authors under the terms of the [Creative Commons Attribution 4.0 License](https://creativecommons.org/licenses/by/4.0/) in the format provided by the authors and unedited. Further distribution of this work must maintain attribution to the author(s) and the published article's title, journal citation, and DOI.

Supplement DOI: <https://doi.org/10.6084/m9.figshare.23954868>

Parent Article DOI: <https://doi.org/10.1364/BOE.498421>

**AUTOUNMIX: AN AUTOENCODER-BASED SPECTRAL UNMIXING METHOD FOR
MULTI-COLOR FLUORESCENCE MICROSCOPY IMAGING: SUPPLEMENTAL
DOCUMENT**

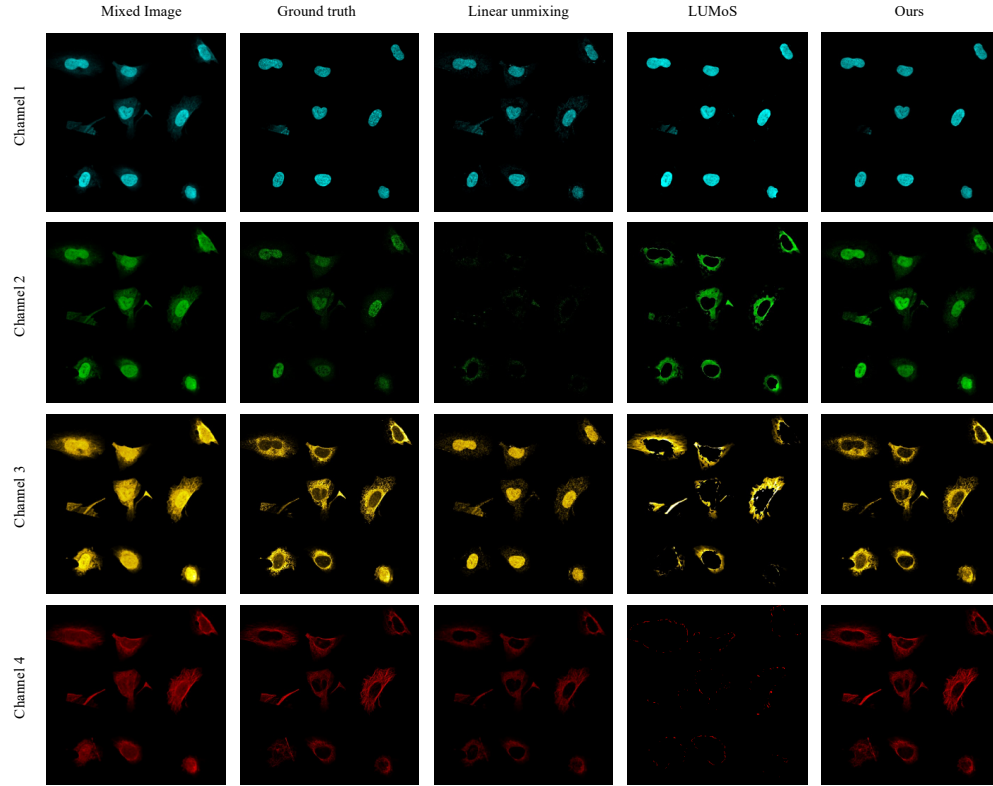


Fig S1 Comparison between linear unmixing, LUMoS, and Ours on 4-channel simulated data of Fig. 2e Each row corresponds to each channel of the images. Columns are mixed image, ground truth, results of linear unmixing, results of LUMoS, and results of Ours. Linear unmixing: MSE 0.00578, SSIM 0.786, PSNR 22.4. LUMoS: MSE 0.00769, SSIM 0.77, PSNR 21.2. Ours: MSE 0.000749, SSIM 0.985, PSNR 31.3

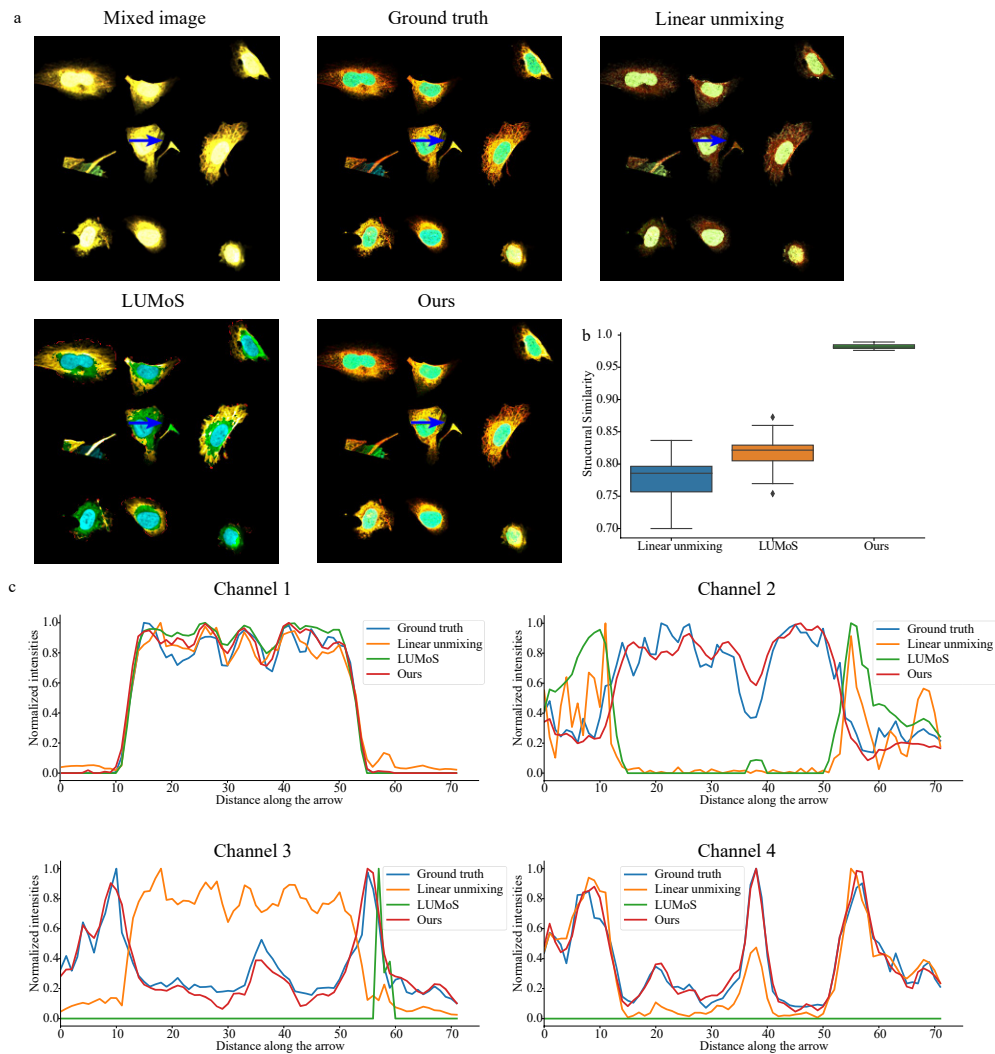


Fig S2 Quantitative comparison between linear unmixing, LUMoS, and Ours on the 4-channel simulated data **a.** Overlay of the mixed image, ground truth, linear unmixing, LUMoS, and Ours in Fig. S1. **b.** Boxplot of SSIM results of linear unmixing, LUMoS, and Ours on the 4-channel simulated data. Our method has highest SSIM with smallest variance. **c.** Normalized intensity values along the blue arrows for each channel in **a**, which shows that our method has high consistency with ground truth compared to other two methods.

Table S1 Quantitative results of blind unmixing performance on datasets with different combinations of fluorophores

	Methods	MSE	SSIM	PSNR
Group 1 (Fig.3a)	Linear unmixing	0.0032 ± 0.0009	0.92 ± 0.014	25 ± 1.16
	LUMoS	0.0032 ± 0.0009	0.91 ± 0.019	25 ± 1.13
	Ours	0.0092 ± 0.0013	0.97 ± 0.0063	30 ± 1.3
Group 2 (Fig.3b)	Linear unmixing	0.0017 ± 0.0004	0.94 ± 0.009	28 ± 0.98
	LUMoS	0.0038 ± 0.0007	0.89 ± 0.016	24 ± 0.79
	Ours	0.0085 ± 0.0012	0.97 ± 0.006	31 ± 1.2
Group 3 (Fig.3c)	Linear unmixing	0.0046 ± 0.0011	0.87 ± 0.023	23 ± 1.05
	LUMoS	0.0054 ± 0.0018	0.82 ± 0.003	22 ± 1.15
	Ours	0.011 ± 0.0014	0.95 ± 0.013	28 ± 0.95

The quantitative results on different combinations of fluorophores are shown in Table S1 corresponding to Fig. 3. Our method achieves SSIM 0.97+ and PSNR 30+ on the generated 3-channel mixed images, and SSIM 0.95 and PSNR 28 for the mixed data of four fluorophores. Linear unmixing and LUMoS both exhibit much worse unmixing performance than our AutoUnmix. The results indicate AutoUnmix has great potential for impressive generalization performance.

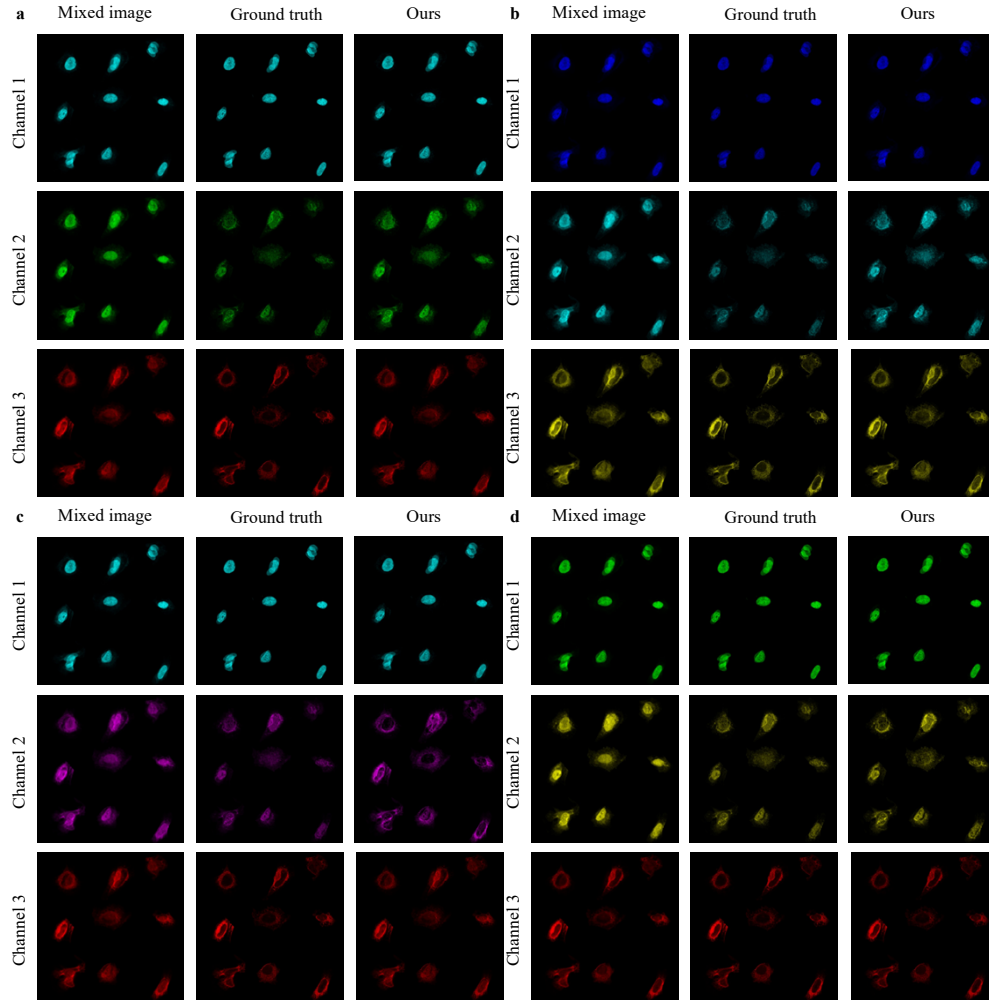


Fig. S3 Results of blind unmixing for Fig. 3a. Each channel of the results is shown in the figures above. We show the unmixing results for the same sample image as in Fig. 3a generated with largely different relative ratio of fluorophore combinations (**a**: CF488, ATTO488, ATTO514, bandpass filter: 515/10, 525/10, 535/10nm; **b**: TagBFP, Cerulean, Citrine, bandpass filter: 450/20, 485/20, 530/20nm; **c**: CF633, CF660R, CF680R, bandpass filter: 650/10, 683/10, 700/10nm; **d**: AzamiGreen, Citrine, mCherry, bandpass filter: 505/20, 530/20, 600/20nm;). All of these images are successfully unmixed with our method with SSIM 0.99+ and PSNR 34+, even under significant different relative ratios.

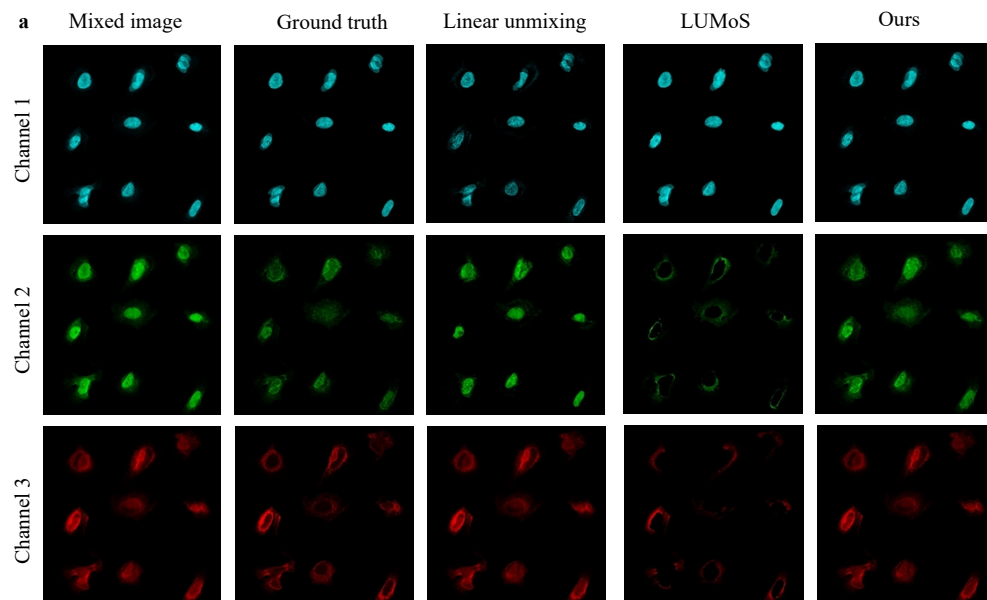


Fig. S4 Comparison of blind unmixing for Fig. 3a. Each channel of the results is shown in the figures above. The mixed image shows channel 1, 2 and 3 are highly overlapped and is consistent with the spectral curve in Fig. 3a. Ours can address this balanced overlapping situation, outputting images almost the same as the ground truth. However, linear unmixing cannot recover some details in channel 2 correctly. LUMoS even cannot reconstruct complete structures in channel 2 and channel 3. Linear unmixing: MSE 0.0024, SSIM 0.93, PSNR 26.3. LUMoS: MSE 0.0032, SSIM 0.89, PSNR 24.9. Ours: MSE 0.0074, SSIM 0.974, PSNR 31.3.

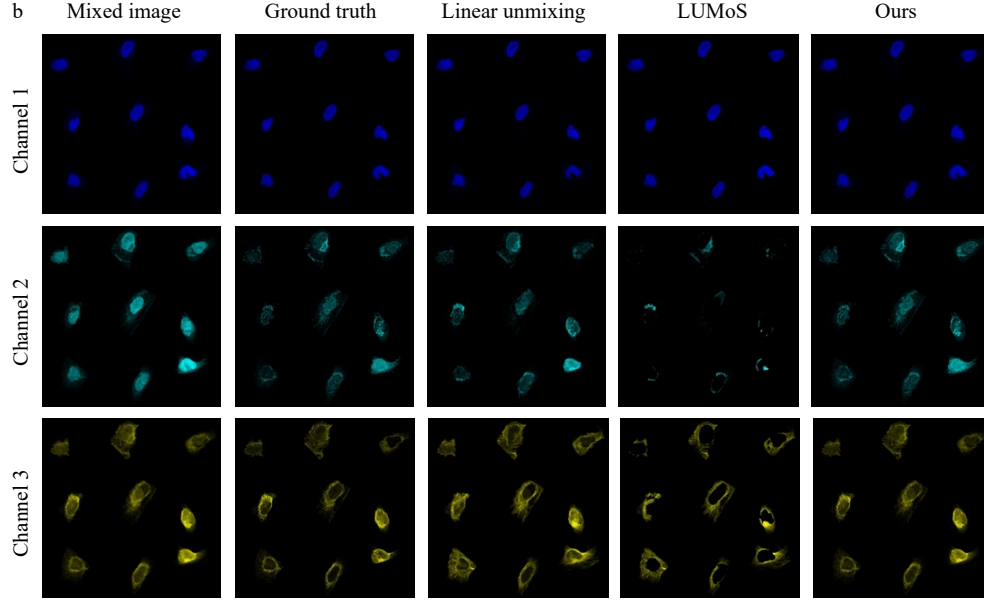


Fig. S5 Comparison of blind unmixing for Fig. 3b. Each channel of the results is shown in the figures above. The mixed image shows channel 2 and channel 3 are highly overlapped and is consistent with the spectral curve in Fig. 3b. Our unmixing output can address such spectra leaks, leading to high consistency with ground truth. However, linear unmixing cannot recover all structures correctly and LUMoS also cannot reconstruct complete structures in channel 2 and channel 3. Linear unmixing: MSE 0.0012, SSIM 0.948, PSNR 29. LUMoS: MSE 0.0029, SSIM 0.91, PSNR 25.4. Ours: MSE 0.0074, SSIM 0.974, PSNR 31.3.

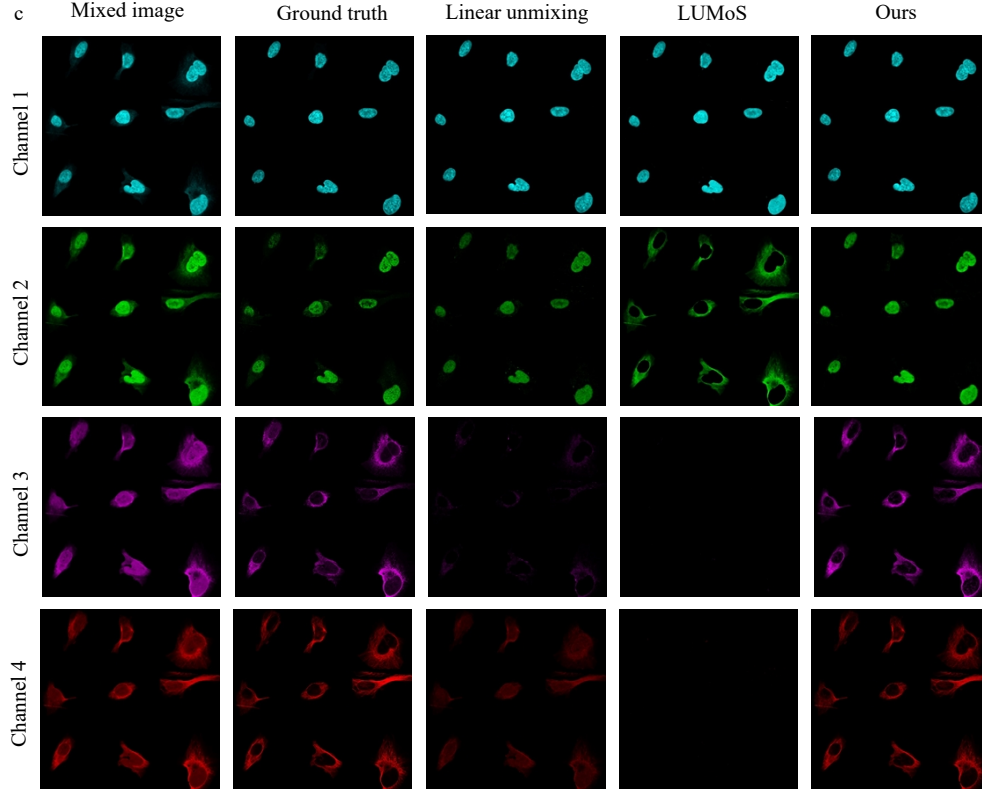


Fig. S6 Comparison of blind unmixing for Fig. 3c. Each channel of the results is shown in the figures above. The mixed image shows channel 2, 3 and 4 are highly overlapped. It is obvious that linear unmixing cannot reconstruct any structures in channel 3. LUMoS performs much worse in channel 2 and channel 4 without correct reconstructions. Compared with ground truth, our results are almost the same and can separate different cell structures explicitly. Linear unmixing: MSE 0.0070, SSIM 0.858, PSNR 21.5. LUMoS: MSE 0.0135, SSIM 0.745, PSNR 18.7. Ours: MSE 0.0093, SSIM 0.976, PSNR 29.4

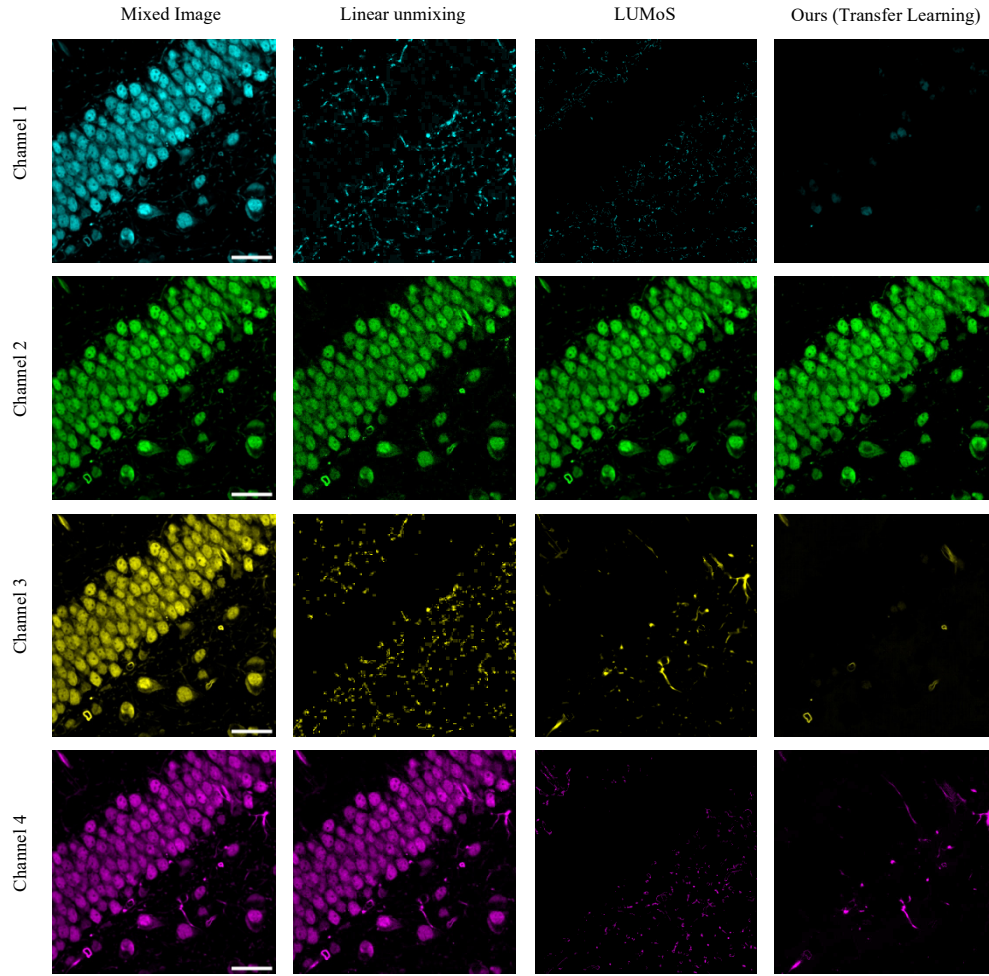


Fig. S7 Comparison between linear unmixing, LUMoS, and Ours on the real 4-channel sample in Fig. 4b. Each row shows the unmixing results of each channel between three methods. Scale bar: 30 μ m. Our method with transfer learning can achieve best unmixing performance. Linear unmixing cannot separate the sample from original highly overlapped images, while LUMoS can separate them but cannot reconstruct complete sample structure in channel 1 and 4.

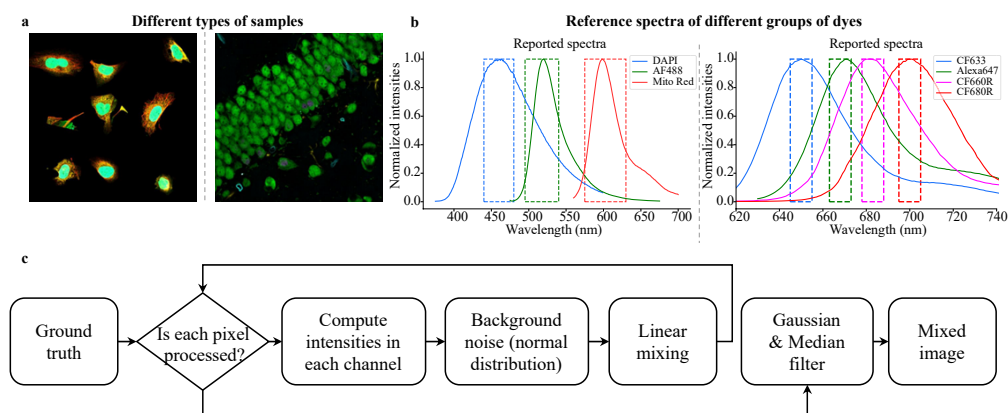


Fig. S8 Generation flow of simulated datasets. **a.** Different multi-channel images selected as ground truth. **b.** Reference spectra of different combinations of dyes. **c.** The generation flow of simulated datasets that translates ground truth into mixed images with the selected sample images and reference spectra using linear mixing. The generation flow corresponds to the description of Simulated datasets in Section Data Acquisition.

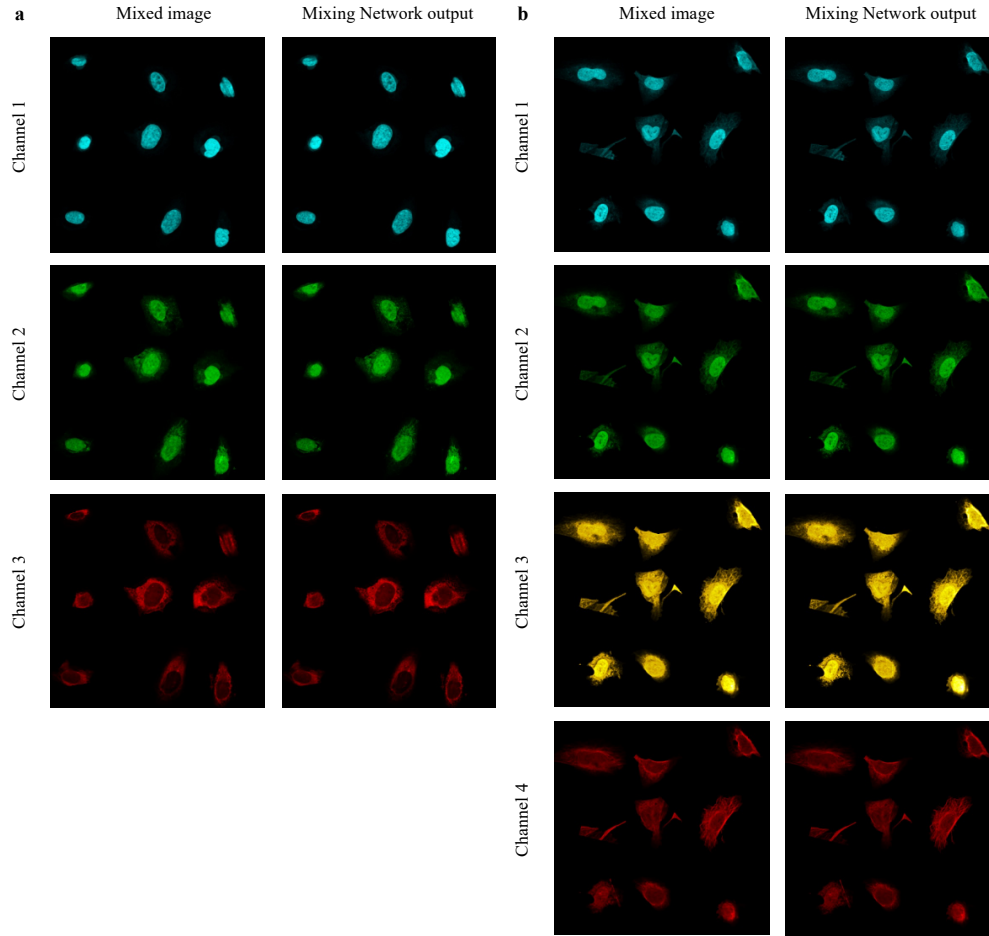


Fig. S9 Results of mixing network. a. 3-channel original mixed image and the output mixed image from the mixing network of our AutoUnmix. MSE: 0.000111, SSIM: 0.998, PSNR: 39.6. **b.** 4-channel original mixed image and the output mixed image from our AutoUnmix. MSE: 0.000171, SSIM: 0.997, PSNR: 37.7. The figure and the quantitative results demonstrate that our method can learn from the spectral mixing process and reconstruct high quality mixed image.

Table S2 Unmixing performance on simulated datasets between U-Net and Ours

		SSIM	PSNR
Group 1	U-Net	0.993 ± 0.0023	34.3 ± 1.15
	Ours	0.997 ± 0.0052	34.5 ± 1.56
Group 2	U-Net	0.98 ± 0.0029	31.1 ± 0.77
	Ours	0.98 ± 0.0035	31.2 ± 0.7
Group 3	U-Net	0.97 ± 0.0065	29 ± 1.3
	Ours	0.97 ± 0.0063	30 ± 1.3
Group 4	U-Net	0.97 ± 0.007	30 ± 1.3
	Ours	0.97 ± 0.006	31 ± 1.2
Group 5	U-Net	0.94 ± 0.012	26 ± 0.77
	Ours	0.95 ± 0.013	28 ± 0.95

To validate that our proposed unmixing network can indeed improve unmixing performance, we also replace the unmixing network in our method with a U-Net. The quantitative results are shown for different simulated datasets tested in our work. Group 1 and group 2 correspond to the 3-channel unmixing and 4-channel unmixing in Fig. 2 respectively. Group 3, 4 and 5 correspond to the blind unmixing validation for different reference spectra in Fig. 3, respectively. We can clearly conclude that our method outperforms the U-Net method in all simulated datasets, though U-Net can achieve close unmixing results to Ours in Group 1 and 2.

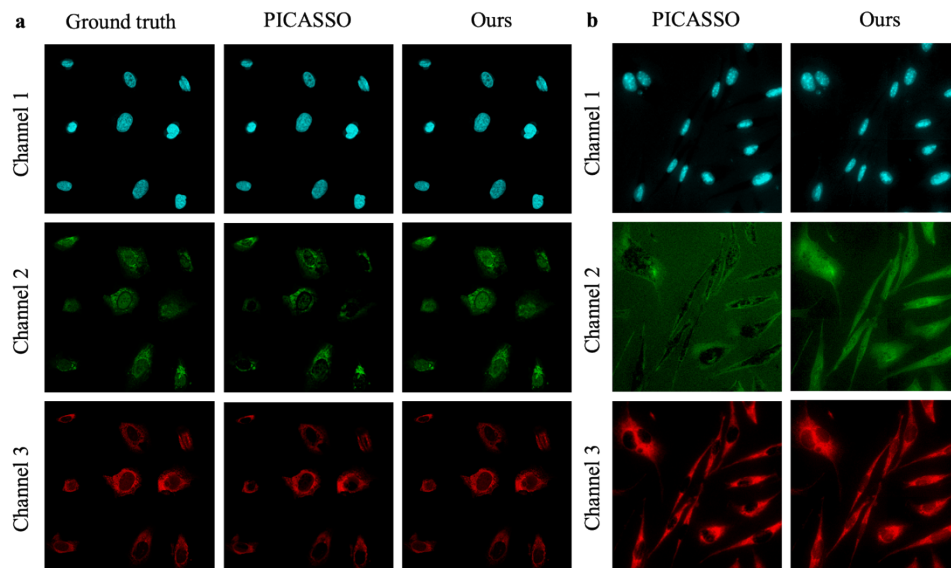


Fig. S10 The visual comparison between ours and PICASSO. a. The unmixed images of ground truth, PICASSO and ours on 3-channel simulated datasets. Each row represents channel 1, 2, and 3 respectively. Both ours and PICASSO can reconstruct images in channel 1. However, PICASSO cannot maintain the cell structure in channel 2 and lose some cell edges in channel 3. Our method performs high consistency with the ground truth. **b.** The unmixed results for real-acquired images comparing PICASSO and ours. The PICASSO method fails to reconstruct the complete structure in channel 2.

Table S3 Quantitative comparison between PICASSO and Ours

	MSE	SSIM	PSNR	Time
Ours (AutoUnmix)	0.0003	0.999	35.4	~60ms
PICASSO	0.0009	0.969	30.5	6.5s

The quantitative comparisons are listed in the table above, which indicating that our AutoUnmix surpasses PICASSO with a large margin. In addition, our AutoUnmix can achieve unmixing within 60ms with the help of RTX 2080 GPU, while PICASSO requires 6.5s to finish this task, which corresponds to the 100-fold improvement in our manuscript. The results in this table and Fig. S10 demonstrate that our AutoUnmix has superior performance over PICASSO in terms of unmixing image quality and algorithm efficiency.

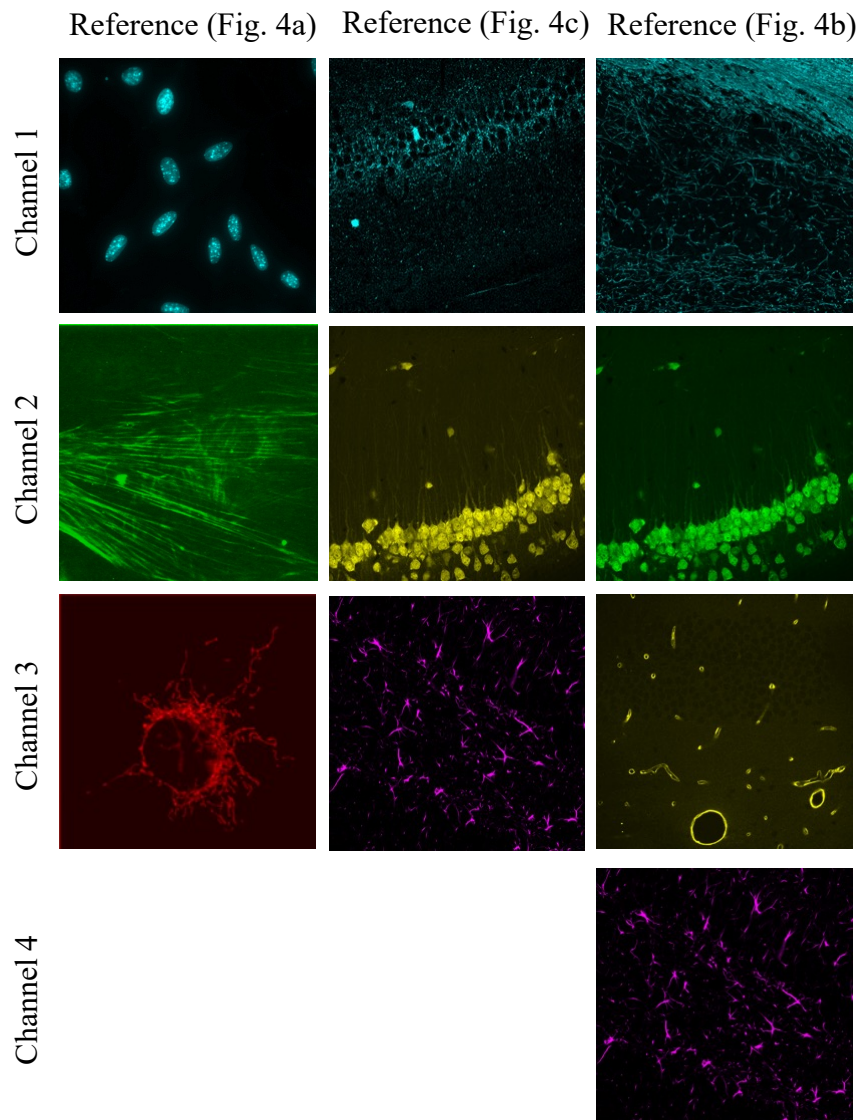


Fig. S11 The reference for experiments on real biological samples. Col 1 shows the typical images of mouse cell nuclei, actin fibers, and mitochondria. Col 2 shows the typical images of PV, NeuN, and GFAP of mouse brain slice. Col 3 shows the typical images of mouse brain slice CNP1, NeuN, GluT1, and GFAP. These images display the morphological features of each tissue and can serve as a reference to discern the fidelity of unmixing method outputs.